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Our Docket No. EU 98055 CON
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MESSAGE:**Appeal No.:** 2007-0091**Serial No.:** 09/765,491**Appellant:** Jack L. Arbiser**Filed:** January 18, 2001**For:** *CURCUMIN AND CURCUMINOID INHIBITION OF ANGIOGENESIS*

Further to the Oral Hearing on February 6, 2007, attached are:

1. **A Submission in Response to Questions Newly Raised During Oral Hearing; and**
2. **Copy of Arbiser, et al, Molecular Medicine, 4:191-195 (1998).**

PTO/SB/21 (09-04)

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Application Number

09/765,491/ Appeal No. 2007-0091

Filing Date

January 18, 2001

First Named Inventor

Jack L. Arbiser

Art Unit

1617

Examiner Name

Jennifer Kim

Attorney Docket Number

EU 98055 CON

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
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- ☐ Amendment/Reply
- ☐ After Final
- ☐ Affidavits/declaration(s)
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- ☐ Reply to Missing Parts/ Incomplete Application
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- ☐ Status Letter
- ☒ Other Enclosure(s) (please identify below):

Submission in Response to Questions Newly Raised During Oral Hearing on February 6, 2007; and copy of Arbiser, et al., Molecular Medicine, 4:191-195 (1998).

Remarks

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Rivka D. Monheit

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48,731

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EU 98055 CON 077113/00004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant: Jack L. Arbiser

Appeal No.: 2007-0091

Serial No.: 09/765,491

Art Unit: 1617

Filed: January 18, 2001

Examiner: Jennifer M. Kim

For: ***CURCUMIN AND CURCUMINOID INHIBITION OF ANGIOGENESIS***

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**SUBMISSION IN RESPONSE TO QUESTIONS NEWLY RAISED DURING ORAL
HEARING ON FEBRUARY 6, 2007**

Sir:

Further to the Oral Hearing on February 6, 2007, Appellant submits the following responses to some of the questions newly raised by the Board during the Oral Hearing. Also enclosed is a copy of Arbiser, *Molecular Medicine*, 4:191-195 (1998), which was discussed during the Oral Hearing. It is believed that no fee is required with this submission. However, should a fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

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Remarks

During the Oral Hearing on February 6, 2007, the Board raised some questions that had not been previously raised by the Examiner or discussed in the Appeal Brief or Reply Brief. Appellant respectfully requests consideration of the following responses to the Board's questions.

Question 1: Claim 4 refers to the treatment of skin disorders and includes lymphangiogenesis in the list of disorders that can be treated. What skin disorders are associated with lymphangiogenesis?

Response: Lymphangiogenesis has been implicated in a number of skin disorders, including Kaposi's sarcoma (see Jussila and Alitalo, "Vascular Growth Factors and Lymphangiogenesis" *Physiol. Rev.* 82, 673-700, 687 (2002), submitted with Appeal Brief), lymphangiomas (see Jussila at page 687-688), neoplasm metastasis, edema, rheumatoid arthritis, and psoriasis. Inflammatory and malignant disorders such as psoriasis, atopic dermatitis and melanoma stimulate lymphangiogenesis through a process involving VEGF-C/VEGFR3, which is distinct from normal angiogenesis (see Jussila at page 693-694). Lymphangiogenesis may be required for maintenance of an inflammatory process by allowing lymphocytes to migrate to inflammation, as well as by providing lymphatic channels for malignant cells to metastasize.

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Question 2: Claim 10 refers to the treatment of symptoms associated with elevated basic fibroblast growth factor (bFGF). What symptoms are associated with elevated levels of basic fibroblast growth factor; and how are these different from symptoms associated with angiogenesis?

Response: Elevated levels of basic fibroblast growth factor (bFGF) are found in patients with a variety of disorders, including those listed in claim 10. A variety of symptoms are associated with elevated levels of bFGF. For example, patients with recessive dystrophic epidermolysis bullosa (RDEB) show scarring, blister formation within different layers of the skin, chronic erosions, chronic severe pruritus, fusion of fingers, esophageal stenosis, and recurrent infection.

As discussed in the specification at least at page 2, lines 16-18, angiogenesis is stimulated by a large number of mechanisms and no one mechanism appears to be the controlling mechanism. Further, as noted in Thaloer, et al., *Cell Growth and Differentiation*, 9:305-312 (1998), a number of different growth factors are involved in angiogenesis, including fibroblast growth factor, vascular endothelial growth factor, and tumor necrosis factor- α (Thaloer, page 307, right col., last para.). Thus, a disclosure that angiogenesis is occurring, does not mean that the subject has elevated basic fibroblast growth factor levels. Additionally, bFGF is involved in a number of cellular processes in addition to angiogenesis, including proliferation, differentiation, and cell survival.

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Question 3: The Board referred to Example 1 and Figure 6A of WO 95/18606 to Aggarwal, and asked if the dosage for curcumin disclosed in this Figure had been calculated and compared to the range defined by claim 10.

Response: Claim 10 specifies that a curcuminoid is administered in combination with a pharmaceutically acceptable carrier, and defines the carrier as an ointment for topical administration containing between one-half percent (0.5%) and five percent (5%) of the curcuminoid or a polymer formulation for implantation.

Example 1 of Aggarwal refers to Figures 1 through 8. Example 1 is entitled "Antiproliferative Effects of Curcumin", and tests the antiproliferative effect of curcumin in cultured cells. The cultured cells are present in a physiological solution to which the curcumin is added (see page 9, lines 28-30). Curcumin was tested in a dosage ranging from 0 to 3 $\mu\text{g}/\text{ml}$. Figures 6A and 6B are graphs of relative cell viability (%) versus curcumin concentration (mg/mL). Relative cell viability is defined at page 4, lines 1-4 as "thymidine incorporation in treated cells over thymidine incorporation in untreated cells multiplied by 100." At a concentration of 0 mg/mL, both Figures 6 A and 6B indicate 100% cell viability. Thus any decrease in cell viability (below 100%), indicates that either cells are dying or proliferation has been inhibited, or a combination thereof. At a concentration of 2 μg of curcumin/ml, Figure 6A indicates 40% relative cell viability of U-251 cells, and Figure 6B indicates about 55% relative cell viability of HUVEC cells. At a concentration of 3 $\mu\text{g}/\text{ml}$, both figures indicate 0% relative cell viability, i.e. all of the cells have died and/or proliferation has ceased. Thus, the thymidine

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incorporation assay used in this Example cannot distinguish the difference between cytotoxicity and antiproliferative effects due to the addition of curcumin to the cell media.

Further, even if this example can be construed as disclosing antiproliferative effects of curcumin, this Example does not disclose or suggest the use of an ointment for topical administration containing between one-half percent (0.5%) and five percent (5%) of the curcuminoid. One must know the density of the composition used in the example to convert Aggarwal's 2 $\mu\text{g/ml}$ composition to a weight percent value. Aggarwal does not specify what cell medium was used, however typical cell media densities are about 1 g/mL. Therefore, assuming the density of the composition was 1 g/mL, the weight percent of the composition would be $2 \times 10^{-4}\%$. This value is much lower, i.e. over 1,000-fold lower, than the lowest value in the range specified in claim 10. Further, the composition used in the cell assays of Example 1, is not an ointment for topical formulation nor is it a polymeric implant.

Additionally, Aggarwal does not disclose the addition of bFGF to the cells and HUVEC cells do not secrete bFGF. Therefore it appears that the results obtained in Example 1 are independent of the presence of bFGF, let alone elevated levels of bFGF.

Thus, Aggarwal does not disclose or suggest the method defined by claim 10 and its dependent claims.

Question 4: One of the rejections on appeal is whether claims 10-12 and 19 are non-obvious under 35 U.S.C. § 103 (a) in view of Arbiser, *et al.*, *J. Amer. Acad. Dermatol.*, 40(6):925-929

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(June 1999) ("Arbiser 1999") in view of Thaloor in combination with Aggarwal. During the Oral Hearing, the Board substituted Arbiser, *et al.*, *Molecular Medicine*, 4:191-195 (1998) ("Arbiser 1998") (copy enclosed) for Arbiser 1999 in the Examiner's rejection and requested comments. As this rejection had not been clearly presented during the written portion of the Appeal, Appellant's representative did not have Arbiser 1998 before her during the Oral Hearing and had to rely on the Board's reading and characterization of this reference.

Response: Arbiser 1998 discloses that over 50% of patients with recessive dystrophic epidermolysis bullosa (RDEB) have elevated levels of bFGF. However, patients with junctional epidermolysis (JEB) and epidermolysis bullosa simplex (EBS) do not have elevated levels of bFGF. Arbiser 1998 suggests that elevated levels of bFGF may contribute to the pathogenesis of RDEB by functioning as a stimulant of collagenase synthesis, by functioning as an angiogenic factor, or by functioning as a keratinocyte mitogen. Further, Arbiser 1998 notes that angiogenesis inhibitors may antagonize the effects of bFGF in RDEB.

Arbiser 1998 does not disclose the use of curcumin for the treatment of the disorders specified in claim 10, let alone that curcumin could be used to treat these disorders due to its anti-angiogenic properties. Arbiser 1998 also does not disclose the use a topical ointment, much less that one that contains curcumin at a concentration of between 0.5% and 5%. Further, Arbiser 1998 does not disclose a polymer formulation containing a curcuminoid for implantation.

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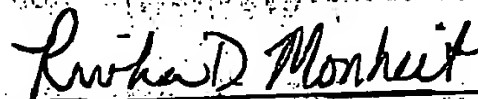
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Submission in Response to Questions Newly Raised during Oral Hearing

It appears that the Examiner is using improper hindsight analysis, using the claims as a blueprint, to select a portion from Arbiser 1999 (or Arbiser 1998), another portion from Thaloor, and a third portion from Aggarwal. However, none of these references, alone or in combination, disclose or suggest the necessary modifications to practice the method defined by claim 10 and its dependent claims.

For the reasons provided in the Appeal Brief, Reply Brief, and presented at the Oral Hearing and in this submission, Appellant submits that the claims are definite, novel and not obvious over the prior art.

Respectfully submitted,



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Date: February 8, 2007

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Basic Fibroblast Growth Factor: A Missing Link between Collagen VII, Increased Collagenase, and Squamous Cell Carcinoma in Recessive Dystrophic Epidermolysis Bullosa

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Communicated by J. Folkman. Accepted February 2, 1998.

Abstract

Background: Patients with recessive dystrophic epidermolysis bullosa (RDEB) have deficiencies of collagen type VII and have elevated levels of fibroblast collagenase, and a greatly increased risk of cutaneous squamous cell carcinoma. Patients with other genetic blistering disorders do not have elevated collagenase or an increased risk of squamous cell carcinoma, despite chronic wounding. The connection between collagen type VII deficiency, increased collagenase, and squamous cell carcinoma is not understood.

Materials and Methods: Urine from 81 patients with RDEB (39 patients), junctional epidermolysis bullosa (JEB; 12 patients), and epidermolysis bullosa simplex (EBS; 30 patients), as well as unaffected family members of RDEB patients (33 patients), was tested for the presence of basic fibroblast growth factor (bFGF) using a sensitive radioimmunoassay. These patients included many who were enrolled in the Epidermolysis Bullosa Registry and others who were referred by their physicians.

Results: Fifty-one percent of patients with RDEB had elevated levels (>5000 pg/g) of urinary bFGF. In contrast, none of the patients with JEB had elevated levels of bFGF. Twenty-one percent of clinically unaffected family members had elevated levels of bFGF, and 13% of patients with EBS had elevated levels of bFGF. The frequency of elevated bFGF values among all groups was statistically significant ($p = 0.002$), and the levels of bFGF in RDEB patients were significantly elevated compared with those of other groups ($p < 0.05$).

Conclusions: We have found that patients with RDEB have elevated levels of bFGF, which may contribute to increased fibroblast collagenase and the development of squamous cell carcinoma. These results suggest a novel treatment for RDEB, namely, angiogenesis inhibitors, which may antagonize the effects of bFGF in this disorder. There are currently no other means of treatment for this disorder, which has a high morbidity and mortality rate.

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is usually a severe and often fatal genetic disease characterized by mechanically fragile skin and subepidermal blistering (1-4). Patients with RDEB have elevated levels of fibroblast-derived collagenase and a high incidence of squamous cell carcinoma, neither of which is observed in the two other major epidermolysis bullosa subtypes, junctional epidermolysis bullosa (JEB) and epidermolysis bullosa simplex (EBS) (5,6). Clinically, RDEB is characterized by a high degree of morbidity from scarring, chronic erosions, chronic severe pruritus, fusion of fingers, esophageal stenosis, recurrent infection, and squamous cell carcinoma (1-4). Patients with all major subtypes of RDEB have greatly diminished levels of collagen type VII. This is a component of anchoring fibrils, which are structures that attach the epidermis to the dermis (7-10). We found that a patient with severe RDEB and bilateral above-the-elbow amputations for recurrent squamous cell carcinoma had elevated levels of urinary basic fibroblast growth factor (bFGF). To assess the role of bFGF in this genetic bullous disease, urine samples from patients with RDEB, JEB, EBS, and unaffected family members were identically screened.

Materials and Methods

Urine samples from patients with RDEB ($n = 39$) and their parents and unaffected siblings ($n = 33$) were analyzed for the presence of bFGF. As potential controls, urine from patients with JEB ($n = 12$) and EBS ($n = 30$) was similarly analyzed. Samples were refrigerated at 4°C during transit and stored at -80°C prior to bFGF radioimmunoassay (11).

A 20-ml aliquot of thawed urine was centrifuged at 2300 rpm for 8 min at 4°C. The supernatant was filtered using a filter with a pore size of 1.2 μ m. The sample was then analyzed using a radioimmunoassay for bFGF according to the manufacturer's instructions (Human bFGF, Quantikine HS, R+D Systems, Minneapolis, MN).

We used the Kruskal-Wallis test to determine overall differences in the levels of bFGF

among EB groups, and Duncan's Multiple Range Test on ranked bFGF values to identify specific differences.

Immunohistochemical Staining

Sections from a bullae from a patient with RDEB were deparaffinized and stained with a rabbit polyclonal antibody against von Willebrand factor (Dako, Carpinteria, CA), at a dilution of 1/100. A secondary mouse anti-rabbit IgG antibody conjugated to diaminobenzidine was incubated at a concentration of 1/50, and the slide was stained according to the manufacturer's directions.

Results

Over 50% of patients with RDEB were found to have levels of urinary bFGF greater than 5000 pg/gram of urine. Normal values of urinary bFGF are less than 5000 pg/g (11). In contrast, none of the patients with JEB had urinary bFGF values greater than 5000 pg/g of urine. Seven out of thirty-three unaffected family members of RDEB had urinary bFGF above 5000 pg/g (21%), and 4 out of 30 patients with epidermolysis bullosa simplex had urinary bFGF levels above 5000 pg/g (13%). The Kruskal-Wallis test showed that there was statistical significance between groups, with one group being significantly different from all other groups ($p = 0.002$). The Duncan's Multiple Range test showed that bFGF values in RDEB significantly differed from those of the other three groups ($p < 0.05$) (Fig. 1, Table 1). In contrast, values from non-RDEB sources were not significantly different from each other. No correlation was seen between the extent of body surface involvement and the presence of squamous cell carcinoma in RDEB patients.

Discussion

Epidermolysis bullosa comprises a heterogeneous group of genetic disorders of the skin characterized by blister formation within different layers of the skin. RDEB has been linked to defects in collagen type VII, and it is usually characterized by severe cutaneous and extracutaneous disease activity and a highly increased risk of cutaneous squamous cell carcinoma (1-6). Whereas both RDEB and JEB can be associated with severe

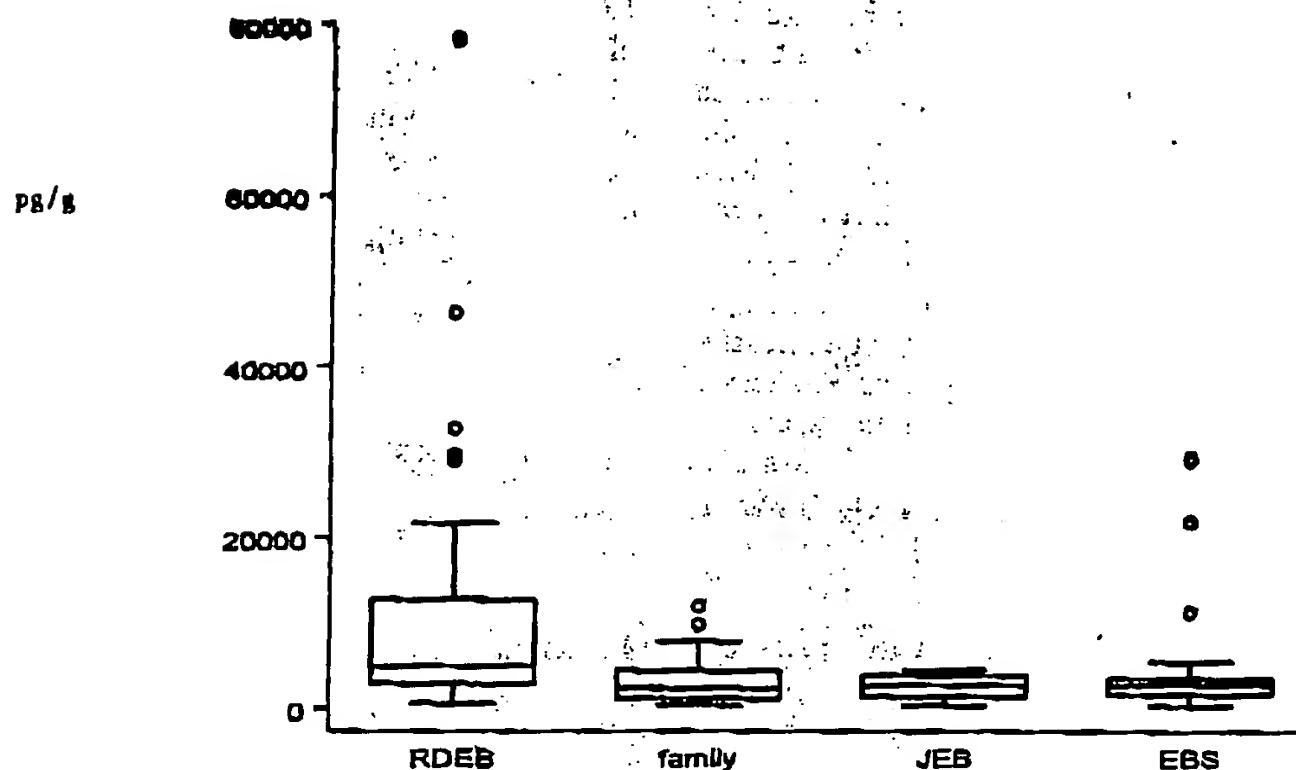


FIG. 1. Comparison of urinary bFGF values between patients with recessive dystrophic epidermolysis bullosa (RDEB), unaffected family members of patients with RDEB (family), junctional epidermolysis bullosa (JEB), and epidermolysis bullosa simplex (EBS). The top dots on the bar graph represent extreme values, the upper

side of the rectangle represents the upper quartile values, the bar inside the rectangle represents the median value, and the lower side of the box represents the lower quartile. The error bars above and below each rectangle represent 1.5 \times the interquartile range drawn towards the nearest data point.

morbidity and mortality, only RDEB is associated with elevated levels of collagenase and an extremely high incidence of cutaneous squamous cell carcinoma. This study provides a potential explanation for these findings.

Prior to the discovery of collagen VII defects in RDEB, these patients had been shown to have elevated collagenase activity both in dermal extracts and in supernatants from fibroblast cultures (12,13). Based on this finding, phenytoin, a weak inhibitor of collagenase, was tested for its ability to ameliorate the symptoms (14-16). Initial studies appeared to demonstrate a beneficial

effect, although a double-blinded, crossover, placebo-controlled larger study failed to confirm any significant overall benefit (17). In addition, no mutations in collagenase genes were discovered in RDEB. While the association between alteration in anchoring fibrils, blister formation, and type VII collagen mutations is apparent, the connection between these mutations and the presence of elevated collagenase and squamous cell carcinomas is not readily apparent.

We have discovered that patients with RDEB have elevations of basic fibroblast growth factor in their urine. Examination of the dermis in a patient with RDEB and elevated bFGF reveals numerous microvessels beneath a typical bulla (Fig. 2). The subepidermal location of bullae at the sites where heparan sulfate proteoglycans bind bFGF may contribute to release of bFGF.

bFGF has several activities that may contribute to the pathogenesis of RDEB. First, it is a stimulant of collagenase synthesis, which may account for the elevated collagenase and perpetuation of blistering observed in these patients (12). bFGF is also a potent angiogenic factor, which may enhance the growth of squamous cell carcinoma (18,19). Finally, bFGF is a keratinocyte mitogen, and chronic proliferative stimuli to

Table 1. Range of urinary bFGF values

Diagnosis	bFGF Values (pg/g)		N (Number of patients)
	Median	Range	
RDEB	5021	609-78297	39
Family	2396	422-11916	33
JEB	2785	351-4604	12
EBS	2830	311-29407	30

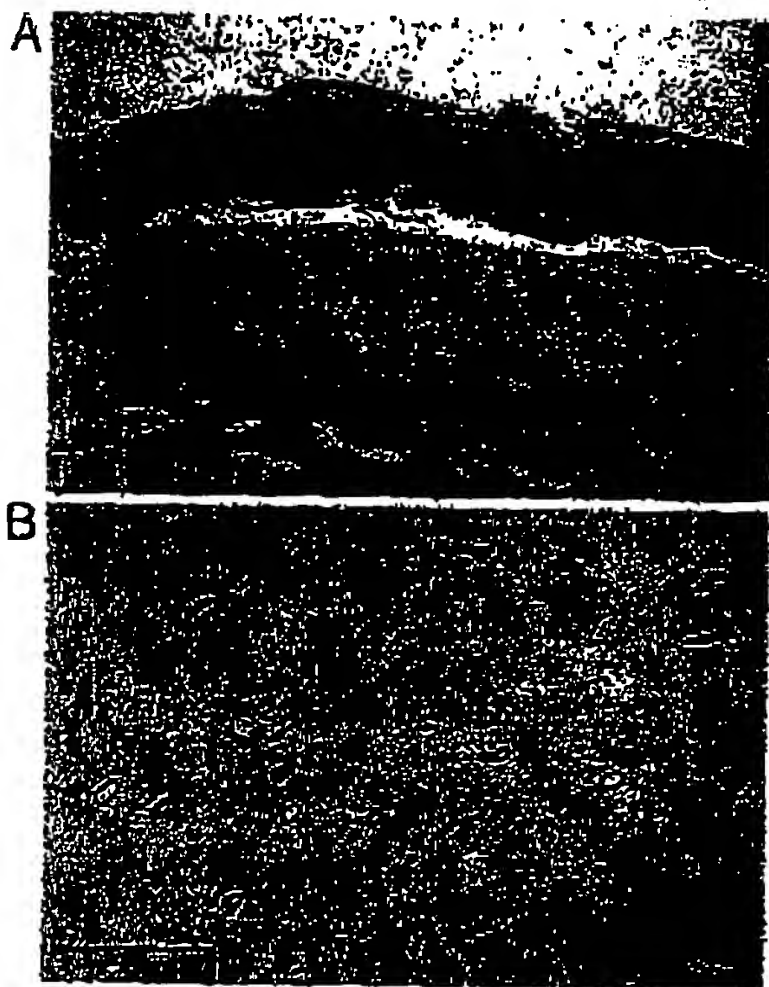


FIG. 2. Histological and immunohistochemical staining of involved tissue in a patient with RDEB. (A) Hematoxylin and eosin stain of a bulla from a patient with RDEB ($\times 100$); (B) von Willebrand staining of the dermis from the same section. The black-stained lumens represent microvessels ($\times 100$). The bars represent 50 μm .

keratinocytes may predispose to squamous cell carcinoma (20). Overexpression of collagenase in the skin of transgenic mice causes an elevated incidence of cutaneous squamous cell carcinoma (21). Thus elevation of bFGF and collagenase may contribute to cutaneous carcinogenesis.

We did not observe a strict correlation between the presence of squamous cell carcinoma and bFGF values. The measurements of bFGF in this study were at a single time point. Continued observation of this cohort over a period of years is needed to determine whether bFGF levels correlate with the eventual development of cutaneous squamous cell carcinoma.

We did not find urinary bFGF elevated in all RDEB patients. We postulate at least two possible explanations. First, bFGF may be present in high levels at skin lesions, and once local binding is saturated, it may enter the systemic circulation. Some of our patients may not have had fully saturated tissue binding sites, resulting in undetectable levels within their urine. Secondly, it is possible that different mutations in collagen type

VII, which cause variable levels of basement membrane disruption, may result in differing rates of release of bFGF. We feel that the release of bFGF is not strictly a function of wounding, however, as patients with JEB have equally severe cutaneous wounding but lack elevated bFGF levels.

These results suggest strategies that may be useful in alleviating the morbidity of RDEB. Inhibition of bFGF in tumor cells by α interferon has been suggested as a potential mechanism for the anti-tumor activity of α interferon, and this activity may be of benefit in RDEB (22). Also, inhibitors of bFGF activity, such as tyrosine kinase inhibitors, may antagonize bFGF-mediated collagenase expression (23). Finally, potent and specific inhibitors of collagenase may be useful in the prevention of bullae formation and scarring (24).

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